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14. ABSTRACT

The goals of the study in the second stage is to investigate vascular oxygenation and tissue oxygen tension of breast tumor under four different hyperbaric oxygen exposures, using near-infrared spectroscopy and FOXY oxygen sensor simultaneously. Hyperbaric oxygenation results in a significant increase in the tumor oxygenation and has superior effect than normobaric oxygen in all seventeen tumors. The improvement of tumor oxygenation could be maintained for 10 to 20 minutes after the end of the hyperbaric oxygen exposure. Multiple correlations were examined between magnitudes of vascular (Δ[HbO2]) and tissue (pO2) responses. Significant correlations were found between responses to normobaric oxygen/carbogen breathing using either modality, but not for responses to hyperbaric oxygen/carbogen. Vascular (R2=0.78) and tissue oxygenation (R2=0.65) also showed the correlations between responses to normobaric and hyperbaric oxygen/carbogen intervention.

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Table of Contents

2005-2006 ANNUAL PROGRESS REPORT

This report presents the specific aims and accomplishments of our breast cancer research project during the first year of funding sponsored by the US Department of the Army. It covers our activities from July 1, 2005 to June 31, 2006.

Introduction

The overall goal of this research project is to apply the multiple monitoring techniques, i.e. Near infrared spectroscopy (NIRS), FOXY oxygen sensor and ^{19}F MR EP imaging of Hexafluorobenzene (HFB), to prove the following hypotheses: combination of hyperbaric oxygen (HBO) intervention can significantly improve breast tumor oxygenation, and that tumor oxygenation remains elevated for a substantial period of time even after HBO exposure, which may be a novel approach to enhance radiosensitivity. If our hypotheses are proven to be true, this study will lead to an optimal intervention plan to improve tumor oxygenation and to determine an optimal time interval after HBO decompression for radiotherapy. Such a novel approach will largely enhance the efficiency of non-surgical therapies for breast tumor treatment and provide a novel prognostic tool for clinical practice. This study will also provide a better understanding of tumor vasculature and tissue oxygen dynamics and spatial heterogeneity under HBO exposure.

The project has three specific aims:

Aim 1: to determine the absolute values of oxygenated hemoglobin concentration, [HbO_2], and hemoglobin oxygen saturation, SO_2, in solid breast tumors from the NIRS measurements.

Aim 2: to investigate vascular oxygenation and tissue oxygen tension of breast tumors under continuous normobaric and hyperbaric oxygen exposures with several gas interventions, using both a single-channel NIRS system and 3-channel FOXY pO_2 system simultaneously.

Aim 3: to investigate global and local dynamics of tumor vascular [HbO_2] and tissue pO_2 of breast tumors immediately after HBO exposure by using both three-channel NIRS and ^{19}F MR EP imaging simultaneously.

Specifically, Task 3 was planned for months 14-30 to accomplish Aim 2.

Task 3: to study the influence of four different gas interventions on tumor oxygenation using both single-channel NIRS and FOXY system, on various rat breast tumor size. (months 14-30)

Body of the report

As mentioned above, the purpose of this project is to investigate vascular and tissue oxygen dynamics in breast tumors by using multiple techniques: NIRS, FOXY and MRI. In the second year, my focus is to study the influence of four different gas interventions on tumor oxygenation using both single-channel NIRS and FOXY system, on various rat breast tumor size (months 14-30). Instead of single-channel NIRS, steady-state diffuse reflectance spectroscopy (SSDRS) was used to measure the vascular oxygen dynamics because it provides the whole spectrum from 500

to 1000nm, which may enable us to calculate both absorption and scattering properties in the future. I monitored breast tumor oxygen dynamics in response to four different gas interventions (the combination of normobaric and hyperbaric oxygen interventions) simultaneously monitored by steady-state diffuse reflectance spectroscopy (SSDRS) and FOXY oxygen sensor.

1. Animal Preparation and experimental setup

Female Fischer 344 rats were used with subcutaneously growing mammary adenocarcinoma 13762NF (originally obtained from DCT, NIH) on the dorsum of the thigh. When the tumors reached ~1 cm in diameter, the rats were anesthetized with ketamine hydrochloride (1.5ml; 100mg/ml; Aveco, Fort Dodge, IA) and xylazine by intraperitoneal injection. Tumor hair was trimmed for the ease of optical contact for transmitting NIR light and FOXY probe insertion. The rats were placed in the hyperbaric chamber on their sides, and then the probes of SSDRS and FOXY were fixed securely on the tumor. Tumor oxygenation parameters were measured simultaneously by SSDRS and FOXY when the rat was exposed to varying gas environment according to the
following challenge paradigms:

1) air-oxygen-hyperbaric oxygen-air;
2) air-carbogen-hyperbaric carbogen-air;
3) air-oxygen-hyperbaric oxygen-oxygen-air;
4) air-hyperbaric air-air.

The chamber was flushed with air for 15 min for baseline measurement, followed by normobaric hyperoxic gas (100% oxygen or carbogen) for 15 min, then followed by a pressure increase to 2 atmospheres absolute for 30 min. The chamber pressure was then reduced to ambient, followed by a flushing with normobaric gas (air or oxygen). Gas exchange (air to oxygen/carbogen) was accomplished at an initial flow rate of approximately 15 l/min. Compression and decompression (from 1 to 2 atmosphere oxygen/carbogen, and 2 to 1 atmosphere oxygen/carbogen) required approximately 2 min. A total of ninteen rats were used in the study: six rats (group 1) were subjected to respiratory challenge 1, five rats (group 2) were exposed to challenge 2, six rats (group 3) were exposed to challenge 3, two rats (group 4) were in challenge 4.

Typical time profiles of $\Delta[HbO_2]$, pO_2 in response to respiratory challenge with hyperoxic gas are shown for a representative 13762NF breast tumor (2.5 cm^3) in Figure 1. This tumor showed a rapid response to oxygen intervention at ambient pressure with a significant increase in $\Delta[HbO_2]$. The three readings in pO_2 displayed heterogeneous responses to normobaric oxygen inhalation. One region had distinct improvement in pO_2, while the other two regions had a little improvement in pO_2. After oxygen was pressured to 2 atmospheres, $\Delta[HbO_2]$ had a further increase, while pO_2 readings in the three respective regions showed significant improvement under the hyperbaric oxygen exposure. Returning to normobaric oxygen from hyperbaric oxygen produced a gradual decline for both $\Delta[HbO_2]$ and pO_2. Three pO_2 reading reach to stabilized level in different rates, but all faster than $\Delta[HbO_2]$. Switch oxygen back to air, both $\Delta[HbO_2]$ and pO_2 has further decline with similar rates.

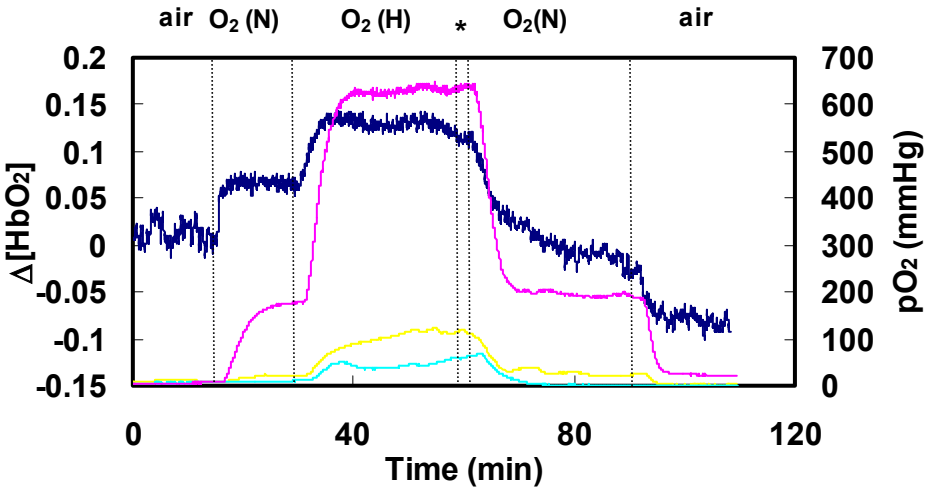

Figure 1 Time course of $\Delta[HbO_2]$ and pO_2 in response to respiratory challenge for a representative tumor, simultaneous measured by SSDRS (blue curve) and FOXY (light blue, pink and yellow curves). O_2 (N) represents normobaric oxygen inhalation, O_2 (H) represents hyperbaric oxygen inhalation. The symbol "*" represents the time needed for decompression.

Tumor oxygen tension has significant improvement in response to normobaric hyperoxic gas intervention ($p<0.01$) for all 17 tumors. Hyperbaric oxygen/carbogen produced further significant increase ($p<0.03$) in pO_2 from normobaric oxygen/carbogen (table 1). For tumors in group 1 and 2, 20 min after the hyperbaric gas was switched back to air, pO_2 was still significantly greater ($p<0.05$) than that in baseline air. However, compared with pO_2 achieved with normobaric oxygen/carbogen before hyperbaric oxygen/carbogen intervention, tumor oxygen tension decreased to a value, which was not significantly higher ($p>0.08$) in 10 min after the gas was switched back to air. Continuing breathing normobaric oxygen after hyperbaric oxygen could not sustain the high tumor oxygen tension achieved with hyperbaric oxygen. Indeed, within 10 min normobaric oxygen after hyperbaric oxygen intervention, pO_2 of tumors in group 3 decreased to a value similar to maximal pO_2 achieved with 15 min normobaric oxygen before hyperbaric intervention ($p=0.5$).

Table 1 Variation of oxygen tension (pO2) in individual rat mammary 13762NF ademocarcinomas in response to gas intervention.

Group	No	Baseline(air)	NBO	HBO	10 min after HBO	15 min after HBO	20 min after HBO
1	6	11.69 ± 2.6	17.5 + 4.0	58.9 + 21.3	21.48 + 4.6	16.89 + 3.1	14.26 + 2.4
2	5	7.42 ± 1.5	17.54 + 3.8	105.6 + 25.7	21.21 + 6.3	14.39 + 3.3	12.36 + 2.3
3	6	13.17+ 2.2	45.15 + 13.2	162.4 + 44.2	44.81 + 13.3	41.7 + 12.8	41.07 + 12.4

There was a strong correlation ($R^2=0.78$) between maximal change of tumor oxygen tension achieved with hyperbaric oxygen/carbogen intervention and that achieved with normobaric oxygen/carbogen intervention in 17 tumors. Among the pO_2 reading in 17 tumors, there is 26% pO_2 reading with greater improvement ($\Delta pO_2 > 5$ mmHg) under hyperbaric hyperoxic exposure than little change ($\Delta pO_2 < 5$ mmHg) during normobaric intervention. The maximal $\Delta[HbO_2]$

achieved with normobaric hyperoxic gas was also correlated with that under hyperbaric hyperoxic gas exposure (R^2=0.65).

There is correlation (R^2=0.52) between change of pO_2 and maximal $\Delta[HbO_2]$ with respect to normobaric hyperoxic gas intervention for 17 tumors (figure 3), while there is no correlation (R^2=0.15) between change of pO_2 and maximal $\Delta[HbO_2]$ with respect to hyperbaric hyperoxic gas intervention.

Hyperbaric air was also tested for the potential usage as a gas intervention to improve tumor oxygenation. In figure 2, it clearly shows that hyperbaric air improve $\Delta[HbO_2]$ but not pO_2.

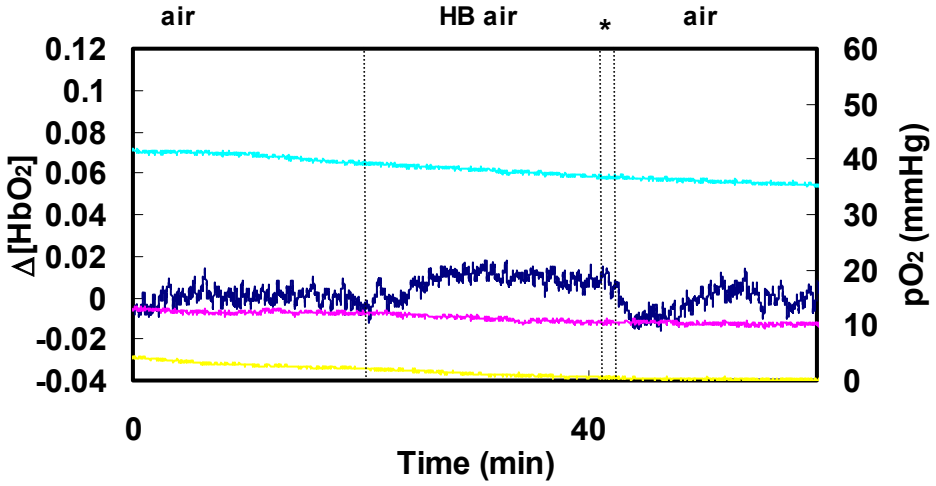

Figure 2 Time course of $\Delta[HbO_2]$ and pO_2 in response to respiratory challenge for a representative tumor with gas intervention sequence air-hyperbaric air-air, simultaneous measured by SSDRS (blue curve) and FOXY (light blue, pink and yellow curves). The symbol "*" represents the time needed for decompression.

Key Research Accomplishments

1) The PI had completed the simultaneous measurement of tumor vascular and tissue oxygen dynamics under four different hyperbaric interventions using multi-channel FOXY oxygen sensors and SSDRS.
2) Multiple correlations were examined between magnitudes of vascular ($\Delta[HbO_2]$) and tissue (pO_2) responses. Significant correlations were found between responses to normobaric oxygen/carbogen breathing using either modality, but not for responses hyperbaric oxygen/carbogen.

Reportable Outcomes:

Peer-reviewed Journal:

1) **Mengna Xia**, Vikram Kodibagkar, Hanli Liu and Ralph Mason, "Tumor oxygen dynamic measured simultaneously by near infrared spectroscopy and ^{19}F magnetic resonance imaging in

rats", Physics in Medicine and Biology,51: 45-60(2006) .

2) **Mengna Xia,** Benjamin Levine, Ralph Mason, Hanli Liu, "Tumor vascular and tissue oxygen dynamics under hyperbaric oxygen exposure", manuscript.

Presentation and Proceeding Papers:

1) **Mengna Xia,** Benjamin Levine, Ralph Mason, Hanli Liu, "Simultaneous monitoring of tumor vascular oxygenation and tissue oxygen tension under hyperbaric oxygen exposure", in Biomedical Topical Meetings on CD-ROM (The Optical Society of America, Washington, DC, 2005).

Conclusions:

Up to date, we could draw the following conclusions from the work that we have conducted in Year 2:

1) Normobaric hyperoxic gas (oxygen and carbogen) can effectively manipulate the vascular oxygen level in breast tumors. However, different regions in a tumor show heterogenous responses to normobaric oxygen inhalation. Hyperbaric oxygen is shown to improve both the tumor vascular and tissue oxygen level more effectively than normobaric oxygen. It is clinically important for the oxygen tension of those tumor regions, which could be significantly improved by hyperbaric oxygen/carbogen, but not by normobaric oxygen/carbogen.
2) The tumor oxygenation 10 min after the end of hyperbaric oxygen exposure is already not significantly higher than oxygenation achieved with normobaric oxygen, which may suggest that having irradiation after HBO exposure may not be superior to performing radiotherapy during normobaric oxygen intervention.

Reference:

Bate T 1969 The treatment of stage 3 carbinoma of the cervix by external radiotherapy and high-pressure oxygen *Br J Radiol* **42** 266-9

Becher A, Kuhnt T, Liedtke H, Krivokuca A, Bloching M and Dunst J 2001 Oxygenation measurements in head and neck cancers during hyperbaric oxygenation *Strahlenther Onkol* **178** 105-8

Becker A, Kuhnt T, Liedtke H, Krivokuca A, Bloching M and Dunst J 2002 Oxygenation measurements in head and neck cancer during hyperbaric oxygenation *Strahlenther Onkol* **2** 105-8

Brady L, Plenk H, Hanley J, Glassburn J, Kramer S and Parker R 1981 Hyperbaric oxygen therapy for carcinoma of the cervix stages IIB, IIIB, and IVA: results of a randomized study by the radiation therapy oncology group *Int J Radiat Onclo Biol Phys* **7** 991-8

Brizel D, Lin S, Johnson J, Brooks J, Dewhirst M and Piantadosi C 1995 The mechanisms by which hyperbaric oxygen and carbogen improve tumour oxygenation *British J of cancer* **72** 1120-4

Bussink J, Kaanders J, Strik A, Vojnovic B and Kogel A v d 2000 Optical sensor-based oxygen tension measurements correspond with hypoxia marker binding in three human tumor xenograft lines *Radiat Res* **154** 547-55
Gu Y, Bourke V A, Kim J G, Constantinescu A, Mason R P and Liu H 2003 Dynamic response of breast tumor oxygenation to hyperoxic respiratory challenge monitored with three oxygen-sensitive parameters *Appl. Opt.* **42** 2960-7
Zhao D, Constantinescu A, Chang C, Hahn E W and Mason R P 2003 Correlation of tumor oxygen dynamics with radiation response of the Dunning Prostate R3327-HI tumor Radiat Res 159 621-31
Zhao D, Constantinescu A, Hahn E W and Mason R P 2001 Tumor oxygen dynamics with respect to growth and respiratory challenge: investigation of the Dunning prostate R3327-HI tumor Radiat. Res. 156 510-20

Appendix:

Tumor vascular and tissue oxygen dynamics under hyperbaric oxygen exposure

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Abstract

We demonstrate the ability to investigate breast tumor oxygen dynamics simultaneously by steady-state diffuse reflectance spectroscopy (SSDRS) and FOXY oxygen sensor in response to normobaric and hyperbaric oxygen intervention. SSDRS directly detected global variations in the oxygenated hemoglobin concentration ($\Delta[HbO_2]$) within tumors and regional oxygen tension (pO_2) were monitored by FOXY oxygen sensor. Multiple correlations were examined between magnitudes of vascular ($\Delta[HbO_2]$) and tissue (pO_2) responses. Significant correlations were found between responses to normobaric oxygen/carbogen breathing using either modality, but not for responses hyperbaric oxygen/carbogen. Vascular (R^2=0.78) and tissue oxygenation (R^2=0.65) also showed the correlations between responses to normobaric and hyperbaric oxygen/carbogen intervention. Hyperbaric oxygenation results in a significant increase in the tumor oxygenation and has superior effect than normobaric oxygen in all seventeen tumors. The improvement of tumor oxygenation could be maintained for 10 to 20 minutes after the end of the hyperbaric oxygen exposure.

Keywords: Near infrared spectroscopy, Breast tumor, oxygenation, hyperbaric oxygen, Hemoglobin

1. Introduction

Tumor hypoxia has proven to have a prognostic impact in cancers and is associated with poor response to radiotherapy (Hall 1994, Zhao *et al* 2003). Hyperbaric oxygenation offers an approach to overcome tissue or tumor hypoxia. Several randomized studies investigating hyperbaric oxygenation as a radiosensitizer were performed 30-40 years ago (Bate 1969, Berry *et al* 1979, Brady *et al* 1981). However, hyperbaric oxygenation was largely abandoned because of the hazard and complexity of irradiation under hyperbaric exposure. Recently, a few groups have developed and demonstrated a new strategy to combine hyperbaric oxygenation and radiotherapy, based on the finding that tumors maintained elevated oxygenation even after exposure to hyperbaric oxygen (Kinoshita *et al* 2000, Hunugita *et al* 2001, Becker *et al* 2002). Accordingly, accurate evaluation of tumor oxygenation in response to hyperbaric intervention could be crucial for determination the optimal time to perform radiotherapy.

Given the importance of tumor oxygenation, several techniques based on microelectrode, magnetic resonance imaging (MRI) have been mainly utilized to monitor the preservation of tumor oxygenation after hyperbaric oxygen exposure (Kinoshita *et al* 2000, Becker *et al* 2002). While each approach has unique strengths, some are highly invasive. Steady-state diffuse reflectance spectroscopy (SSDRS) is an effective technique for determining the optical absorption and scattering coefficients of biological tissues and other turbid media (Farrell *et al* 1992, Kienle and Patterson 1997, Nichols *et al* 1997). It has been developed in recent years as a promising non-invasive technique to determine the concentration of tissue chromophores, such as oxygenated and deoxygenated hemoglobin (Hull *et al* 1998, Doornbos *et al* 1999). However,

SSDRS currently lacks spatial resolution, and thus, the utility of global measurements require validation, given the well-documented heterogeneity of tumor oxygenation. Therefore, Multiple fiber-optic probes may be used to monitor regional oxygen tensions in tumors (Griffiths 1999, Bussink *et al* 2000, Zhao *et al* 2001), to help us better understanding of the interplay of vascular and tissue oxygenation.

We now applied two monitoring techniques simultaneously, i.e., steady-state diffuse reflectance spectroscopy to monitor tumor vascular oxygenation and a multi-channel FOXY oxygen sensor to measure regional tumor oxygen tension when the rats were under a sequence hyperoxic gas intervention including hyperbaric oxygen. Moreover, we also measured the preservation of tumor oxygenation after hyperbaric oxygen exposure and compare it with the tumor oxygenation achieved with normobaric oxygen intervention. The objective of study was to investigate breast tumor oxygen dynamics in response to hyperbaric oxygenation and to examine whether a possible improvement in tumor oxygenation is maintained after hyperbaric oxygenation.

2. Materials and Methods

2.1 Animal Preparation and experimental setup

Female Fischer 344 rats were used with subcutaneously growing mammary adenocarcinoma 13762NF (originally obtained from DCT, NIH) on the dorsum of the thigh. When the tumors reached ~1 cm in diameter, the rats were anesthetized with ketamine hydrochloride (1.5ml; 100mg/ml; Aveco, Fort Dodge, IA) and xylazine by intraperitoneal injection. Tumor hair was trimmed for the ease of optical contact for transmitting NIR light and FOXY probe insertion.

The rats were placed in the hyperbaric chamber on their sides, and then the probes of SSDRS and FOXY were fixed securely on the tumor. Tumor oxygenation parameters were measured simultaneously by SSDRS and FOXY during respiratory challenge with hyperbaric oxygen (HBO) or carbogen (HBCB).

During the experiments, the rats were exposed to hyperbaric oxygen in an acrylic hyperbaric chamber. The chamber was flushed with air for 15 min, followed by normobaric 100% oxygen or carbogen for 15 min, then followed by a pressure increase to 2 atmospheres absolute for 30 min. The chamber pressure was then reduced to ambient, followed by a flushing with air or oxygen. Gas exchange (air to oxygen/carbogen) was accomplished at an initial flow rate of approximately 15 l/min. Compression and decompression (from 1 to 2 atmosphere oxygen/carbogen, and 2 to 1 atmosphere oxygen/carbogen) required approximately 2 min. A total of seventeen rats were used in the study: six rats (group 1) were subjected to respiratory challenge in a sequence of air-oxygen-hyperbaric oxygen-air, five rats (group 2) breathed air-carbogen-hyperbaric carbogen-air, six rats (group 3) breathed air-oxygen-hyperbaric oxygen-oxygen-air.

2.2 Steady-state diffuse reflectance spectroscopy (SSDRS) for measuring changes in tumor vascular oxygenation (ΔHbO_2)

The instrument used to acquire reflectance spectra from tumor tissue is a broadband diffuse reflectance spectrometer. Briefly, continuous wave (CW) light from a 20 W tungsten-halogen light source (HL-2000HP, ocean optics, FL) is coupled into a 2.6-mm core diameter fiber optic bundle, the distal end of which is placed in physical contact with the surface of the tumor. After

being scattered in the tumor tissue, the transmitted light is collected by a 1-mm core diameter detection fiber, the end of which is coupled to a hand-held spectrometer (USB2000, Ocean optics, FL). The broadband light diffuse spectrometer provides reflectance spectra from 400 to 900 nm.

According to the modified Beer-Lambert law, as given in Eqs. (1) and (2), changes of oxy- and deoxy-hemoglobin concentration, $\Delta[HbO_2]$ and $\Delta[Hb]$, can be derived from the measured amplitudes at two wavelengths (750nm and 830nm), by using extinction coefficients of oxy- and deoxy-hemoglobin published by Cope (Cope 1991).

$$\log(\frac{A_b}{A_t})^{750} = (\varepsilon_{Hb}{}^{750}\Delta[Hb] + \varepsilon_{HbO_2}{}^{750}\Delta[HbO_2]) \cdot DPF \cdot d \quad (1)$$

$$\log(\frac{A_b}{A_t})^{830} = (\varepsilon_{Hb}{}^{830}\Delta[Hb] + \varepsilon_{HbO_2}{}^{830}\Delta[HbO_2]) \cdot DPF \cdot d \quad (2)$$

where A_b is the baseline amplitude, A_t is the transient amplitude during the intervention, and d is the direct source-detector separation. *DPF* (differential path-length factor) is a tissue-dependent parameter and defined as the ratio between the optical path length and the physical separation between the source and detector.

2.3 FOXY oxygen sensor for measuring oxygen tension of tumors (pO_2)

Simultaneously, regional pO_2 values in tumors were monitored by a multi-channel, fiber optic oxygen sensor (FOXY, Ocean Optics Inc, Dunedin, FL). Three fluorescence-quenched, optical fiber probes (AL300, tip diameter 410 μm) were inserted into different regions of the tumors. Light from a pulsed blue LED (475 nm) was coupled into one branch of a bifurcated optical fiber probe and propagated to the probe tip. The distal end of the probe is coated with a thin layer of a hydrophobic sol gel material, in which oxygen-sensing ruthenium complex is

effectively trapped. Illumination of the ruthenium complex causes fluorescence at ~600 nm. If the excited ruthenium complex encounters oxygen molecule, the excess energy is transferred to the oxygen molecule, thus quenching the fluorescence signal. The degree of quenching correlates with the oxygen concentration, pO_2.

3. Results

Typical time profiles of $\Delta[HbO_2]$, pO_2 in response to respiratory challenge are shown for a representative 13762NF breast tumor (2.5 cm^3) in Figure 1. This tumor showed a rapid response to oxygen intervention at ambient pressure with a significant increase in $\Delta[HbO_2]$. The three readings in pO_2 displayed heterogeneous responses to normobaric oxygen inhalation. One region had distinct improvement in pO_2, while the other two regions had a little improvement in pO_2. After oxygen was pressured to 2 atmospheres, $\Delta[HbO_2]$ had a further increase, while pO_2 readings in the three respective regions showed significant improvement under the hyperbaric oxygen exposure. Returning to normobaric oxygen from hyperbaric oxygen produced a gradual decline for both $\Delta[HbO_2]$ and pO_2. Three pO_2 reading reach to stabilized level in different rates, but all faster than $\Delta[HbO_2]$. Switch oxygen back to air, both $\Delta[HbO_2]$ and pO_2 has further decline with similar rates.

Tumor oxygen tension has significant improvement in response to normobaric hyperoxic gas intervention ($p<0.01$) for all 17 tumors. Hyperbaric oxygen/carbogen produced further significant increase ($p<0.03$) in pO_2 from normobaric oxygen/carbogen (table 1). For tumors in group 1 and 2, 20 min after the hyperbaric gas was switched back to air, pO_2 was still significantly greater ($p<0.05$) than that in baseline air. However, compared with pO_2 achieved

with normobaric oxygen/carbogen before hyperbaric oxygen/carbogen intervention, tumor oxygen tension decreased to a value, which was not significantly higher ($p>0.08$) in 10 min after the gas was switched back to air. Continuing breathing normobaric oxygen after hyperbaric oxygen could not sustain the high tumor oxygen tension achieved with hyperbaric oxygen. Indeed, within 10 min normobaric oxygen after hyperbaric oxygen intervention, pO_2 of tumors in group 3 decreased to a value similar to maximal pO_2 achieved with 15 min normobaric oxygen before hyperbaric intervention ($p=0.5$).

There was a strong correlation ($R^2=0.78$) between maximal change of tumor oxygen tension achieved with hyperbaric oxygen/carbogen intervention and that achieved with normobaric oxygen/carbogen intervention in 17 tumors (Figure 2A). By examing point by point in figure 2, there is 26% pO_2 reading with greater improvement ($\Delta pO_2 > 5$ mmHg) under hyperbaric hyperoxic exposure than little change ($\Delta pO_2 < 5$ mmHg) during normobaric intervention. The maximal $\Delta[HbO_2]$ achieved with normobaric hyperoxic gas was also correlated with that under hyperbaric hyperoxic gas exposure ($R^2=0.65$, figure 2B).

There is correlation ($R^2=0.52$) between change of pO_2 and maximal $\Delta[HbO_2]$ with respect to normobaric hyperoxic gas intervention for 17 tumors (figure 3), while there is no correlation ($R^2=0.15$) between change of pO_2 and maximal $\Delta[HbO_2]$ with respect to hyperbaric hyperoxic gas intervention (figure not shown).

4. Discussion

In the present study, global average $\Delta[HbO_2]$ was measured by SSDRS, and regional pO_2 were obtained simultaneously by a multi-channel fiber optic oxygen-sensing system FOXY, in

response to hyperbaric hyperoxic gas intervention. We used transmission mode NIRS in order to interrogate deep tumor tissue. Two oxygen-sensitive indicators displayed similar dynamic tendency in response to gas interventions.

The simultaneous measurements demonstrate that the two techniques, i.e., SSDRS and FOXY, are consistent and complementary with one another for tumor oximetry. Both systems are relatively inexpensive and provide real-time measurements. Our data demonstrates that oxygenation parameters measured from both techniques show significant and consistent elevation in tumor oxygenation during both normobaric and hyperbaric hyperoxic gas interventions. As expected, $\Delta[HbO_2]$ increased much faster than pO_2 in all tumors, indicating that change in tumor vascular oxygenation precedes tumor tissue oxygenation. The current data also showed that $\Delta[HbO_2]$ and ΔpO_2 in response to normobaric hyperoxic gas intervention are correlated (figure 3). Both observations are consistent with our previous studies in the same tumor type measured simultaneously by NIRS and ^{19}F MRI (Xia *et al* 2006), as well as by NIRS and fiber-optics probes (Gu *et al* 2003). However, $\Delta[HbO_2]$ and ΔpO_2 in response to hyperbaric hyperoxic gas intervention are not significantly correlated. It suggests that the further improvement of pO_2 during hyperbaric gas intervention is contributed to the increased amount of dissolved oxygen molecule in plasma, not those oxygen molecule transported by hemoglobin.

Previous studies have demonstrated that hyperbaric hyperoxic gas intervention improved tumor oxygen tension in mammary adenocarcinomas (R3230Ac), while normobaric oxygen and carbogen did not change tumor oxygenation significantly (Brizel *et al* 1995). In our tumor model, both normobaric and hyperbaric oxygen/carbogen improves the tumor oxygenation (table 1). Moreover, hyperbaric oxygen/carbogen results in much more significant improvement in

tumor oxygenation than normobaric oxygen/carbogen. It is clinically important for the oxygen tension of those tumor regions, which could be significantly improved by hyperbaric oxygen/carbogen, but not by normobaric oxygen/carbogen.

Preservation of pO_2 after hyperbaric oxygen in tumors has been recently studied by several groups (Kinoshita *et al* 2000, Becher *et al* 2001). Their results indicate that tumor oxygen decreased gradually and remained at a high level tens of minutes after HBO exposure, while NBO group showed no significant change after NBO exposure. We also observed that an improvement in tumor tissue oxygenation achieved by hyperbaric oxygenation may be maintained over 10-20 minutes even after the end of hyperbaric oxygenation. However, a major concern is whether the preserved level of the tumor oxygenation after the end of HBO is significantly higher than the tumor oxygenation achieved with normobaric oxygen intervention. Indeed, we found that the tumor oxygenation 10 min after the end of hyperbaric oxygen exposure is already not significantly higher than oxygenation achieved with normobaric oxygen, which may suggest that having irradiation after HBO exposure may not be superior to performing radiotherapy during normobaric oxygen intervention.

In summary, by studying tumor vascular oxygenation concomitantly with changes in tumor oxygen tension, we found several correlations for both modalities under sequences of hyperoxic gas intervention with hyperbaric oxygen exposure. This study also demonstrates the feasibility of conducting simultaneous SSDRS and FOXY oxygen sensor under hyperbaric oxygen exposure. We believe both tumor vascular and tissue oxygenation can provide valuable insights into tumor pathophysiology and response to intervention.

Acknowledgements

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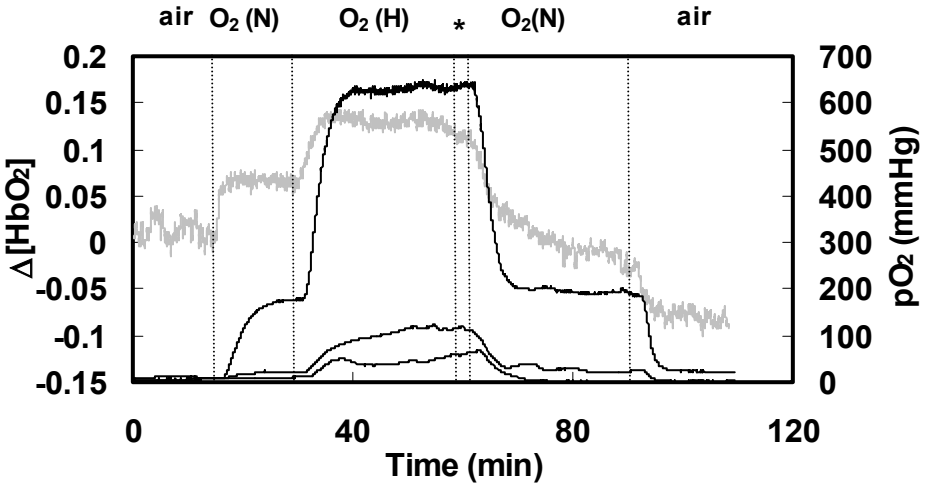

Figure 1 Time course of $\Delta[HbO_2]$ and pO_2 in response to respiratory challenge for a representative tumor, simultaneous measured by SSDRS (thicker and lighter curve) and FOXY (thinner and darker curves). O_2 (N) represents normobaric oxygen inhalation, O_2 (H) represents hyperbaric oxygen inhalation. The symbol "*" represents the time needed for decompression.

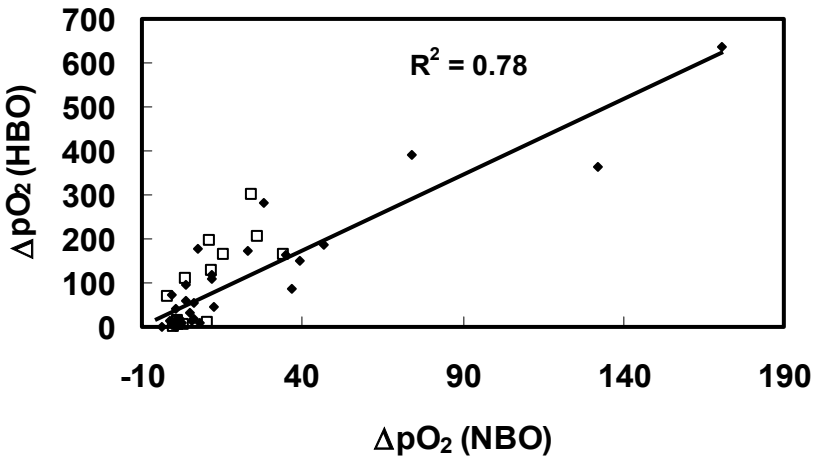

Figure 2A Correlation between maximal change of tumor oxygen tension during normobaric oxygen inhalation and during hyperbaric oxygen inhalation for tumors with oxygen (◆) or carbogen (□)intervention.

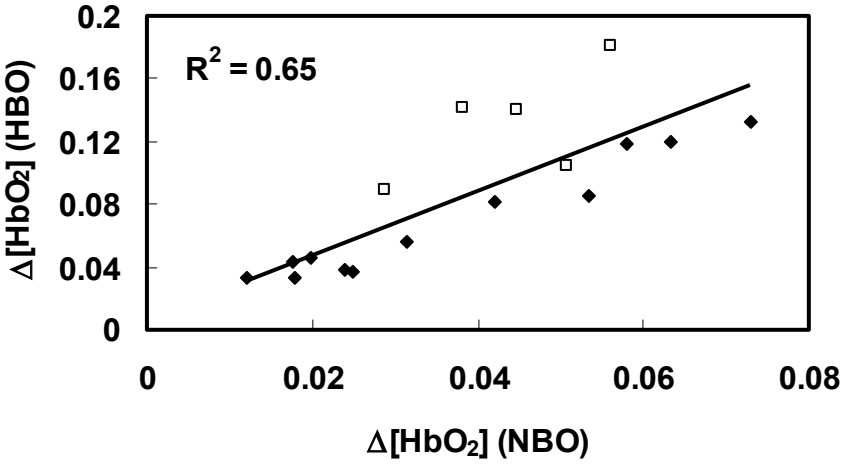

Figure 2B Correlation between maximal change of [HbO_2] during normobaric oxygen inhalation and during hyperbaric oxygen inhalation for tumors with oxygen (◆) or carbogen (□)intervention.

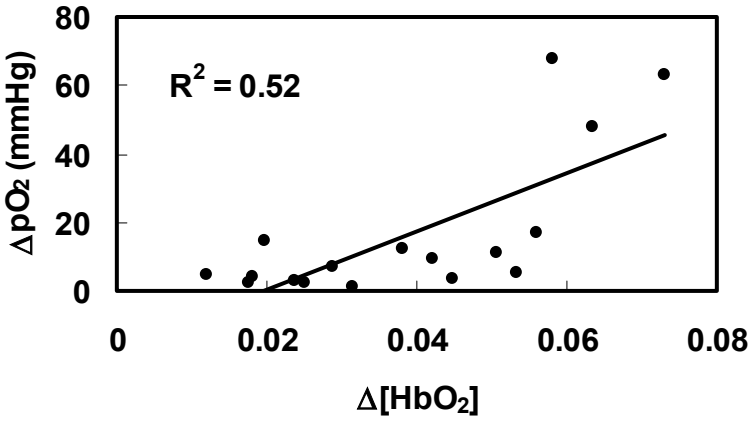

Figure 3 Correlation between change of pO2 and maximal $\Delta[HbO_2]$ with respect to normobaric hyperoxic gas intervention for 17 tumors. The unit for $\Delta[HbO_2]$ is mM/DPF.

Table 1 Variation of oxygen tension (pO_2) in individual rat mammary 13762NF ademocarcinomas in response to gas intervention.

Group	No	Baseline(air)	NBO	HBO	10 min after HBO	15 min after HBO	20 min after HBO
1	6	11.69 ± 2.6	17.5 + 4.0	58.9 + 21.3	21.48 + 4.6	16.89 + 3.1	14.26 + 2.4
2	5	7.42 ± 1.5	17.54 + 3.8	105.6 + 25.7	21.21 + 6.3	14.39 + 3.3	12.36 + 2.3
3	6	13.17+ 2.2	45.15 + 13.2	162.4 + 44.2	44.81 + 13.3	41.7 + 12.8	41.07 + 12.4

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